

THE INTERRELATIONSHIPS
OF BARK BEETLES AND BLUE-STAINING FUNGI
IN FELLED NORWAY PINE TIMBER

BY

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(Contribution from Minnesota Agricultural Experiment Station)

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THE INTERRELATIONSHIPS OF BARK BEETLES AND BLUE-STAINING FUNGI IN FELLED NORWAY PINE TIMBER¹

By J. G. LEACH, *associate plant pathologist and botanist*, L. W. ORR, *instructor in forest entomology*, and CLYDE CHRISTENSEN, *assistant forest pathologist*, Minnesota Agricultural Experiment Station²

INTRODUCTION

It is a well-known fact that trees injured by fire or heavy defoliation, or those felled by wind or in the course of lumbering operations, are very susceptible to attack by bark beetles, wood borers, and fungi. Such timber deteriorates rapidly and is often rendered almost worthless in one season. This fact is very important in forestry operations where it becomes necessary to leave cut logs in the woods for a season or more before they are taken to a mill.

The rapidity with which such timber may decline has been pointed out by Boyce (1),³ who made a study of the deterioration of western yellow-pine trees felled in connection with control of the western pine beetle. He showed that the sapwood of such trees was completely blue-stained by the end of the first season after felling. Decay was also very rapid, especially in the sapwood, but was more pronounced the second season. However, Boyce did not mention any relationship between the bark beetles and the development of the stains and decay.

Graham (6), in a study of the felled tree trunk as an ecological unit, found that there was a definite succession of insects and fungi in the log as the chemical and physical character of the wood changed during the process of disintegration and decay. He pointed out that along with the insects typical of each region and stage of decomposition of the log there also were fungi equally typical of the parts where they occur. He further observed (6, p. 399) that "In some cases there is a distinct symbiotic relationship between wood inhabiting insects and fungi, as in the case of the ambrosia beetles; and in many other instances a looser type of symbiosis can be demonstrated." The details of these relationships, however, were not studied. The work reported in the present paper is the result of one phase of a project planned to study in some detail the interrelations of certain insects and fungi attacking felled logs kept under more or less controlled conditions.

PLAN OF EXPERIMENTS

The field experiments, initiated in the spring of 1931, were carried out at the Lake Itasca Forest Forestry Biology Station, Arago, Minn. Because of its long, smooth bole and relative freedom from

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² The writers wish to express their appreciation to Dr. Louise Dossdall for assistance in identifying some of the fungi and to Vera Koerper for assistance in the histological studies and for the drawings made from them.

³ Reference is made by number (italic) to Literature Cited, p. 340.

limb scars, Norway pine, *Pinus resinosa* Ait., was chosen for the study. Healthy standing trees of suitable size were selected and felled on May 18 and 19. The trunks were immediately cut into logs 40 inches long and 7 to 12 inches in diameter. These logs, after treatment as indicated below, were placed in position under a slanting roof made of laths spaced so as to provide approximately 50 percent shade. The logs were placed upon wooden supports so that the north end was about 6 to 8 inches and the south end about 4 inches above the ground. The wooden supports had previously been painted with a copper



FIGURE 1.—A log from series C, sealed and enclosed in an 18-mesh screen-wire cage.

sulphate and linseed-oil paint as a precaution against spread of decay from the supports to the logs.

The logs were divided into 6 series of 8 logs each, except the check series, in which there were 16 logs. Each series was handled differently, as follows:

- A. Not caged; no treatment (check series).
- B. Not caged; ends and limb scars disinfected with a 2-percent aqueous solution of ethyl mercury chloride, then sealed with roofing pitch and covered with burlap.
- C. Same as series B but enclosed in a cage of 18-mesh aluminum-coated screen wire (fig. 1).
- D. No end treatment but enclosed in a cage of 18-mesh screen wire.
- E. Not caged; ends and limb scars sprayed at frequent intervals throughout the summer seasons with a 2-percent aqueous solution of ethyl mercury chloride.
- F. Same as series E but enclosed in a cage of 18-mesh screen wire.

It was planned that series A should serve as a check series, duplicating as nearly as possible the conditions in nature, being exposed to normal insect attack and any fungus spores disseminated by them as well as to infection by wind-blown spores through the exposed ends and limb scars.

Series B was designed to eliminate infection by wind-blown spores but to leave the logs exposed to normal insect attack. Thus, any fungus infection would be due to inoculum introduced by insects or entering through holes made by the insects.

In series C elimination of both fungus infection and insect attack was attempted.

Series D was planned to eliminate insect attack but to leave the logs exposed to infection by wind-blown spores.

Since logs sealed with pitch and burlap, as in series B and C, would obviously maintain a higher moisture content than unsealed logs and since this treatment might influence the results, the ends of the logs in series E and F were sprayed with a solution of ethyl mercury chloride but not sealed. This treatment, as was determined later, effectively prevented infection through the exposed ends and did not interfere with the normal drying of the logs.

One-fourth of the logs of each series were to be removed for examination at the end of each summer for 4 successive years. In this way the progress of insect attack and fungus infection under the different conditions could be followed and any associations between insect and fungus could be detected.

A record was kept of the particular tree from which each log was cut and the logs were so distributed in each series that any differences in results due to differences in individual trees would be evident. Each log was weighed at frequent intervals throughout the summer in order to determine the variations in weight.

In addition to the experiment outlined above, other experiments were started from time to time in which freshly cut logs were treated and caged, as in series C and F, and into which individual species of insects were introduced. In this way logs sealed against infection by wind-blown spores were subjected to attacks by individual species of insects. Thus the association between a given insect and fungus could be studied without being complicated by the presence of other insects or fungi.

Throughout the course of the investigation the progressive development of the insects and fungi was observed in logs that were barked and cut up at suitable intervals. Cultures of fungi were made from the logs and from the insects in various stages of development. Also insects in various stages of development were killed and embedded in paraffin for histological examination in order to determine the more intimate relationships between the insects and associated fungi. In this way it was hoped to gain a fairly accurate picture of the association of the insects and the fungi involved in the deterioration of the logs.

Two species of bark beetles, *Ips pini* Say and *I. grandicollis* Eich, were among the first insects to attack the exposed logs. This paper presents the results of a study of these two beetles and the fungi associated with them in their development in the logs.

LITERATURE RELATING TO BARK BEETLES AND BLUE STAIN

Apparently the bluing of timber was first described in 1878 by Hartig (8), who recognized its fungal nature and also mentioned the presence of insects in trees affected with blue stain. Münch (12), MacCallum (11), Wilson (20), and others in later years observed the association of blue stain with insect injury, but, apparently, made no study of the nature of the association. Von Schrenk (19), in 1903,

investigated more closely the relation of insects to the spread of blue stain. In addition to describing in detail the development of blue stain in and about the tunnels of *Dendroctonus ponderosae* Hopk., in trees of the western yellow pine, *Pinus ponderosa* Lawson, Von Schrenk dissected a number of beetles and attempted to isolate the blue-stain fungus from the intestinal tract and from feces. He failed to obtain the blue-stain fungus but found a characteristic bacterium present. He considered these trials "by no means conclusive for they were not exhaustive" and more work on a larger scale was projected, but the results were never published. The blue-stain fungus described by Von Schrenk was *Ceratostomella pilifera* (Fr.) Wint., and Rumbold (17, p. 848) is of the opinion that "possibly *C. pilifera* was a secondary blue stain in the wood that he used for starting his cultures and that the fungus that first stained it had lost its vitality by the time the wood reached the laboratory." This error and his failure to isolate the fungus from the beetles probably are responsible for Von Schrenk's conclusion (19, p. 18) that

The spores of the "blue" fungus are probably blown about by the wind in countless thousands, and at the time of the beetle attack in July and August some of the spores lodge in the holes made in the bark of the living pine tree by the bark and wood-boring beetles.

After Von Schrenk's work in 1903 very little attention was given to this insect and fungus association until 1928, when Craighead (5) called attention to the constant association between tree-killing bark beetles (*Dendroctonus*) and blue stain. Craighead pointed out that the girdling of the trees by the bark beetles was not sufficient to cause the rapid death of the trees and suggested a symbiotic relation between bark beetle and blue-stain fungus in which the later contributed to the death of the tree. This was soon followed by the experiments of Nelson and Beal (15) and the more extensive studies of Nelson,⁴ which showed that the girdling of pines by the tunnels of the bark beetles was insufficient to account for the rapid death of the tree. He showed by experimental inoculations that the blue-stain fungi would kill pines in a relatively short time without the help of the beetles, death being caused by the stoppage of sap flow through the stained wood. Nelson was able to isolate the blue-stain fungus from the bodies of beetles, and he concluded that it was commonly introduced under the bark by them.

Using material furnished by Craighead, as well as other material collected from various parts of the United States, Rumbold (17) found that *Ceratostomella pini* Münch was constantly associated with the attacks of *Dendroctonus frontalis* Zimm. and *D. brevicornis* Lec., and that a new species, *C. ips* Rumbold, was associated with *Ips grandicollis* and *I. calligraphus* Germ.

None of the above-named authors studied in detail the nature of the symbiosis between the beetles and the blue-stain fungi. However, a recent paper by Grosmann (7), who was working in Germany, deals in considerable detail with the nature of the symbiosis between bark beetles and fungi. She pointed out that the bark beetles carry yeasts as well as the blue-stain fungi, a fact also demonstrated by

⁴ NELSON, R. M. EXPERIMENTS WITH BLUESTAIN FUNGI IN SOUTHERN PINES. Thesis, Ph.D., Univ. Minn. 1930. (In press.)

Person (16). Grosmann concluded that the insects are in no way dependent upon either the blue-stain fungi or the yeasts. They were considered as commensals.

RESULTS OF THE EXPERIMENTS

EXPERIMENTAL EVIDENCE THAT BLUE-STAIN FUNGI ARE INTRODUCED INTO THE LOGS BY BARK BEETLES

In the study of the relation of an insect to the spread and development of a disease, it is the authors' opinion that, in order to prove conclusively that an insect is an agent of dissemination and inoculation, the disease should be produced experimentally by visitation of the insect under controlled conditions with adequate checks. Although frequent or constant association of the insect with the disease may leave little doubt as to the role played by the insect, the proof is not complete until the relationship has been experimentally demonstrated. Insofar as the writers know this has not been done for the bark beetles and blue stain. One of the objects of these experiments was to determine to what extent bark beetles are dissemination and inoculation agents of blue stain.

The exposed logs that were not caged were found to be infested by bark beetles the latter part of May, only a few days after they were cut and put into position. The development of these and other beetles and the associated fungi was studied by frequent examination of extra logs exposed for that purpose.

On September 18, 1931, approximately 4 months after the experiment was started, the first set of 16 logs was removed and examined. The bark was removed from each log and the degree of insect infestation and the amount of blue stain and other fungus infection were noted. In doing this a 5-inch section of each end of the 40-inch log was disregarded, all notes being made on the central 30 inches. Since there was some variation in the size of the logs, the surface area of this region was recorded for each log. The degree and nature of insect and fungus infestation of each log are given in table 1. Additional evidence confirming the data given in table 1 was obtained from similar logs opened in the two following years and from logs opened while studying the progressive development of beetles and fungi, but since the evidence of association is so clear cut the detailed notes are given for one series only.

It will be seen from table 1 that blue stain always followed the attack of the 2 species of *Ips* and was absent from the logs that were protected from the insects by wire cages. Also, in those logs that became infested with only a few bark beetles that managed to get into the cages, the stain was limited to the immediate vicinity of the *Ips* tunnels, while the remainder of the log was free from stain. One log from each of the six different treatments was photographed, and these are shown in figure 2. Of particular interest is the log representing series D, in which is shown one nuptial chamber of *I. grandicollis* with one short side channel. Observe that blue stain has developed around this tunnel but is absent elsewhere. In figure 3 are shown cuts into logs from series E and F showing the development of blue stain in logs infested with *Ips* and the absence of stain in logs free of insect infestation.

TABLE 1.—Degree and nature of insect infestation and fungus infection of the logs 4 months after the beginning of the experiment

Log no.	Treatment and other data	Insect infestation	Fungus infection	Remarks
1	Not caged; no end treatment; surface area 735 square inches; tree no. 5.	<i>Ips pini</i> , 11 broods; <i>I. grandicollis</i> , 7 broods; <i>Monochamus</i> sp., 11 tunnels; <i>Rhagium</i> sp., 3 larvae; buprestid larvae, 2; a few small unidentified tunnels.	Blue stain abundant, but overgrown by dense mycelial mats of <i>Peniophora gigantea</i> ; <i>Ceratostomella</i> perithecia scarce; yeast and a few unidentified fungi present; some green mold (<i>Trichoderma koningi</i>) in older tunnels; bark very loose.	<i>Peniophora</i> apparently entered through exposed ends, and by rapid invasion of sapwood inhibited the normal development of blue stain.
2	Not caged; no end treatment; surface area 787.5 square inches; tree no. 7.	<i>I. pini</i> , 7 broods; <i>I. grandicollis</i> , 12 broods; <i>Monochamus</i> sp., 8 tunnels; buprestid larvae, 2.	Essentially the same as no. 1.	Essentially the same as no. 1.
3	Not caged; no end treatment; surface area 712.5 square inches; tree no. 5.	<i>I. pini</i> , 18 broods; <i>I. grandicollis</i> , 8 broods; <i>Monochamus</i> sp., 12 tunnels; buprestid larvae, 3; <i>Rhagium</i> sp., 2 larvae.	do.	Do.
4	Not caged; no end treatment; surface area 682.5 square inches; tree no. 3.	<i>I. pini</i> , 18 broods; <i>I. grandicollis</i> , 7 broods; <i>Monochamus</i> sp., 17 tunnels; buprestid larvae, 5; <i>Rhagium</i> sp., 3 larvae.	do.	Do.
7	Not caged; ends and limb scars sealed with pitch and burlap; surface area 817.5 square inches; tree no. 2.	<i>I. pini</i> , 43 broods; <i>I. grandicollis</i> , 17 broods; <i>Monochamus</i> sp., 15 tunnels; <i>Rhagium</i> sp., 3 larvae; buprestid larvae, 30.	Blue stain and yeast abundant and closely associated with <i>Ips</i> tunnels; other fungus infection insignificant or absent; bark loose.	Moisture content of the log much greater than that of logs with ends not sealed.
8	Not caged; ends and limb scars sealed with pitch and burlap; surface area 697.5 square inches; tree no. 6.	<i>I. pini</i> , 51 broods; <i>I. grandicollis</i> , 4 broods; <i>Monochamus</i> sp., 17 tunnels; buprestid larvae, 11.	do.	Do.
9	Caged; ends and limb scars sealed with pitch and burlap; surface area 712.5 square inches; tree no. 6.	None.	No blue stain present; 2 small spots of inner bark stained with a fungus forming small black sclerotia but no spores; apparently entering through minute cracks in the bark; bark not loose.	Do.
10	Caged; ends and limb scars sealed with pitch and burlap; surface area 705 square inches; tree no. 2.	do.	No blue stain or other fungus infection; 100 percent clean; bark not loose.	Do.
11	Caged; no end treatment; surface area 735 square inches; tree no. 2.	<i>I. grandicollis</i> , 3 broods.	Blue stain present only about tunnels of <i>I. grandicollis</i> . Some infection with a white basidiomycete (<i>P. gigantea</i>) entering through exposed ends; bark not loose.	One end of the cage was closed with muslin, a method which proved not to be always proof against smaller beetles; slower development of <i>Peniophora</i> as compared to logs 1-4 probably due to absence of <i>Ips</i> .
12	Caged; no end treatment; surface area 742.5 square inches; tree no. 7.	<i>I. grandicollis</i> , 3 broods, which developed only to egg-channel stage; <i>Pistodes</i> sp., 17 pupal chambers.	Blue stain and yeast associated with <i>Ips</i> egg channels but absent elsewhere; small amount of infection by <i>Peniophora</i> near exposed ends; bark not loose.	Do.
13	Not caged; ends and limb scars sprayed at intervals with ethyl mercury chloride; surface area 840 square inches; tree no. 5.	<i>I. pini</i> , 31 broods; <i>I. grandicollis</i> , 10 broods; <i>Monochamus</i> sp., 10 tunnels; buprestid larvae, 11; <i>Rhagium</i> sp., 4 larvae.	Blue stain and yeast abundant; spreading and fruiting to within 1 inch of ends, where it was checked by spray applied to end of log; some <i>Trichoderma</i> and other secondary fungi present; stain associated with <i>Monochamus</i> and buprestid tunnels only when these made contact with <i>Ips</i> tunnels; bark loose.	Spray applied at intervals to end of logs very effective against fungus infection, at the same time allowing the log to dry as rapidly as logs with no end treatment.
14	Not caged; ends and limb scars sprayed at intervals with ethyl mercury chloride; surface area 795 square inches; tree no. 7.	<i>I. pini</i> , 29 broods; <i>I. grandicollis</i> , 11 broods; <i>Monochamus</i> sp., 11 larvae; buprestid larvae, 5; <i>Rhagium</i> sp., 1 larvae.	do.	Do.
15	Caged; ends and limb scars sprayed at intervals with ethyl mercury chloride; surface area 832.5 square inches; tree no. 7.	None.	One small spot of stain associated with crack in bark; fungus not identified, but not <i>Ceratostomella</i> ; bark not loose.	Do.
16	Caged; ends and limb scars sprayed at intervals with ethyl mercury chloride; surface area 825 square inches; tree no. 5.	<i>I. grandicollis</i> , 4 broods, all on upper side of log.	Blue stain and yeast associated with <i>Ips</i> tunnels but absent elsewhere on log; bark not loose.	These beetles apparently entered through the end of cage covered with muslin.

As will be discussed in more detail later, cultures were made of the fungi found in these and certain other logs. It is sufficient to say here that blue-staining fungi were always found developing and spreading from the bark-beetle tunnels and that the evidence justifies the conclusion that they were introduced into the logs by the beetles and that they were rarely or never introduced in any other way. In addition to the blue-staining fungi, a characteristic yeast was found constantly associated with the beetle attacks. Several different kinds of bacteria, also, were isolated from freshly made tunnels, but these were not consistently present. In the older tunnels several

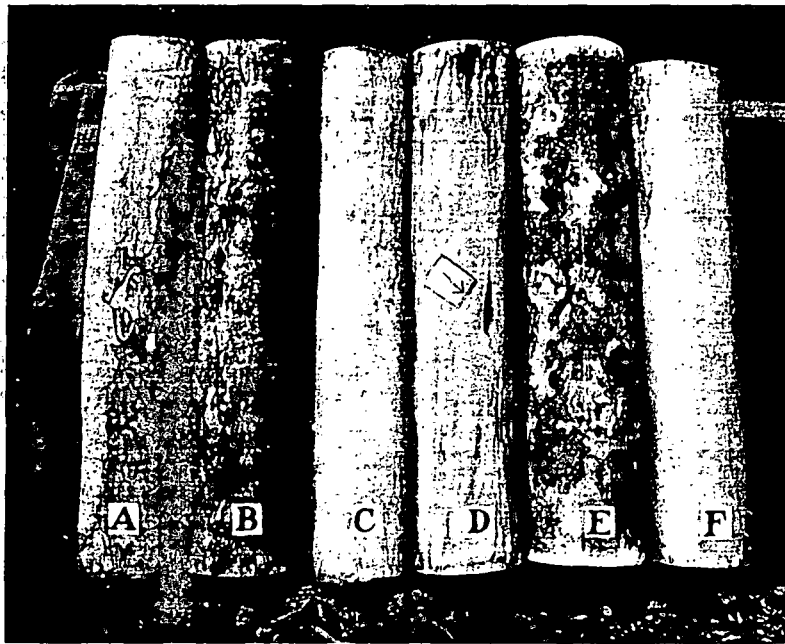


FIGURE 2.—One log from each of the six series; the arrow on the log from series D points to an area of blue stain surrounding an incomplete tunnel made by a single *Ips* beetle that managed to get through the cage.

other fungi were often present, the most common of which was a species of *Trichoderma*. It is very probable that these were also introduced by beetles or other insects, but they develop more slowly than the blue stain and are secondary in nature, apparently not being able to penetrate extensively into the woody tissue. The most common fungus entering through the exposed nontreated ends of the logs was *Peniophora gigantea* (Fr.) Massee. This fungus was not present in any of the logs with sealed or treated ends, but developed abundantly under the loosened bark of the logs with exposed ends and caused a rapid decay of the sapwood. Fruiting bodies were often found on the underside of the infected logs. Sporophores of this and other fungi sometimes grew out through holes made in the bark by insects, but there was no evidence that they were consistently introduced by bark beetles.

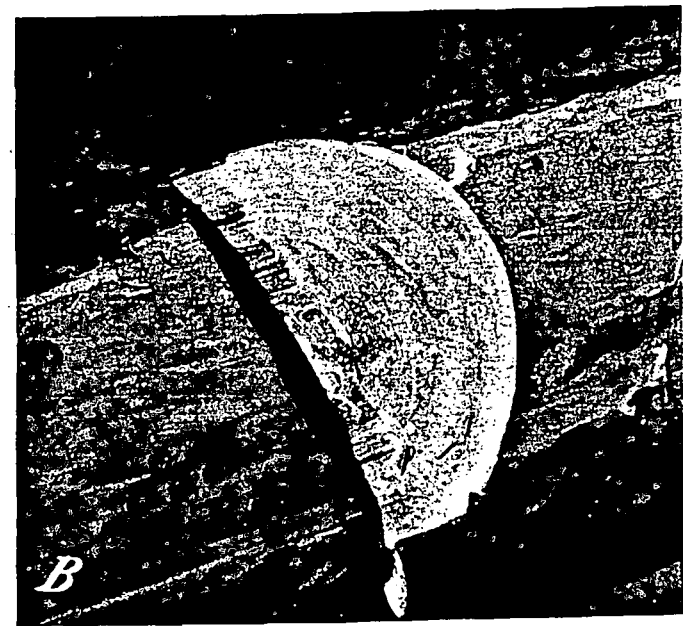
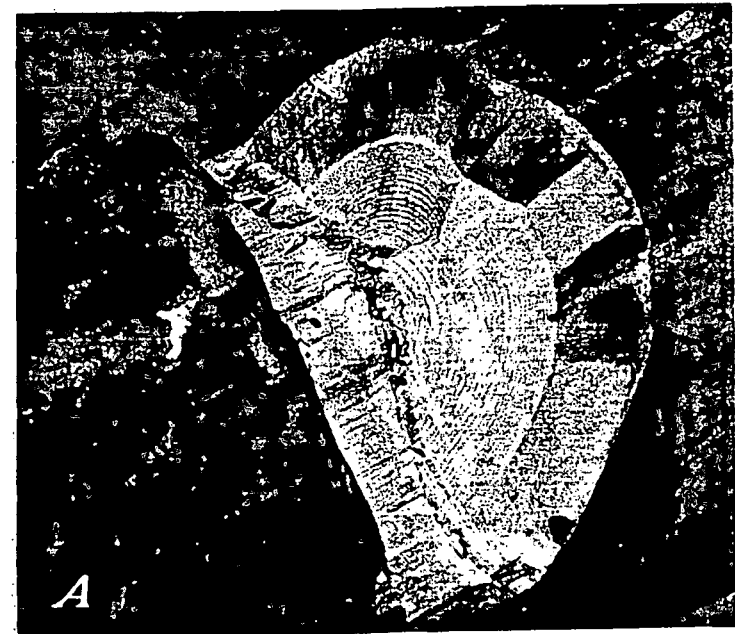


FIGURE 3.—Two logs cut to show presence or absence of stain in the sapwood: A, A log from series E, not caged; B, a log from series F, caged.

As a further check on the association of blue-stain infection and bark-beetle infestation, a log of each series was sawed in cross section as shown in figure 4. With the aid of a planimeter the area of blue-

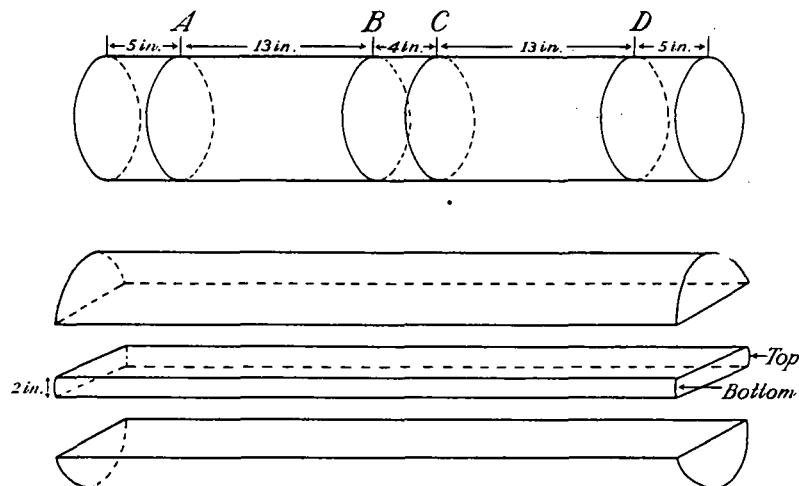


FIGURE 4.—Diagram to show how logs were cut for the measurement of the amount of stained sapwood.

stained wood, showing in the cross sections A, B, C, and D, was measured and calculated in terms of percentage of total area of sapwood in cross section. A plank 2 inches thick was sawed from each of the other logs as shown in figure 4. In the same manner the area

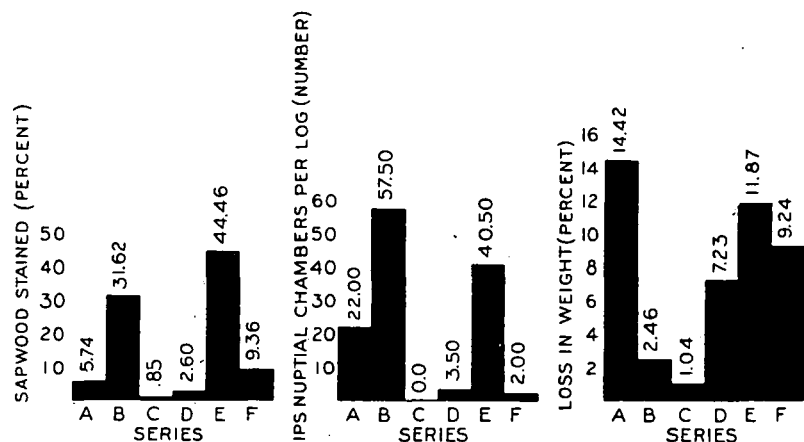


FIGURE 5.—Amount of blue stain in the logs of the different series, and the relation of insect infestation and moisture content of the wood to the prevalence of blue stain.

of stained wood, as revealed in longitudinal section, was calculated. The results of these measurements and also the degree of beetle infestation are shown graphically in figure 5. It will be seen that the amount of stain is closely correlated with the amount of bark-beetle infestation, except in the check logs 1, 2, 3, and 4. The extremely small amount of stain found in these logs that were heavily attacked by bark beetles was difficult to understand until it was noted that

the wood-rotting fungus *Peniophora gigantea* had penetrated practically all of the sapwood and, by its very rapid development, had inhibited the normal development of blue stain. This explanation of the absence of blue stain in these logs was verified the following season by observing the development of the fungi in similar logs at frequent intervals throughout the season.

Since it has been shown that the development of blue stain is influenced by the moisture content of the wood (4, 13) and since the loss of water from the logs was influenced by the different treatments, the logs were weighed at frequent intervals until they were opened. Figure 5 shows graphically the percentage of loss in weight by the logs during the first summer of the experiment. This loss, for practical purposes, may be considered to be due almost entirely to loss of water. As a further check on the moisture content of the logs, six increment borings were made from a log of each series just before the bark was removed for examination. The approximate percentage of moisture in each sample (oven-dry basis) was as follows: A, 69; B, 110; C, 102; D, 70; E, 55; and F, 58. Although the nature of this determination is subject to considerable error, the relative values agree closely with the data obtained from the loss of weight. Since blue stain develops well at any moisture content between 55 and 110 percent, it is improbable that moisture content was a limiting factor in blue-stain development in these experiments. It is obvious from the data given in figure 5 that there is no correlation between the amount of blue stain and the moisture content of the logs of the different series.

Because of the several kinds of insects that entered the uncaged logs and the resulting overlapping of their tunnels, it was often difficult to interpret with assurance the significance of the fungi found associated with the tunnels. In the case of the bark beetles this difficulty was in part overcome by the fact that a few beetles entered the cages of logs 11, 12, and 16, which resulted in these logs being infested with a few isolated broods. In order further to overcome this difficulty additional logs were caged, with ends sealed or sprayed, and into each cage a few beetles of a single species were introduced. In all of these logs blue stain developed abundantly about all of the *Ips* tunnels and was absent on other parts of the log, verifying the conclusion that the blue-stain fungi are commonly introduced into the logs only by the bark beetles.

ASSOCIATION OF BARK BEETLES AND FUNGI

Parallel with the experiments reported above, the more intimate relationships between bark beetles and fungi were studied. Logs infested with bark beetles were opened at frequent intervals and the progress of fungus infection determined by microscopic and cultural studies. At the same time, insects in all stages of development were killed, fixed, and embedded in paraffin and were later studied by histological methods. In this manner, a fairly complete picture of the association between the bark beetles and the fungi was obtained. For the sake of brevity the results of both studies are presented together and the association is described in chronological sequence following the life cycle of the insects.

As stated in the foregoing, two species of bark beetles, *Ips pini* and *I. grandicollis* attacked the logs soon after they were cut and put in

place. Because of the similarity of development and fungus association of the two species, they will be discussed together, any observed differences being pointed out as the occasion demands.

The life history of these bark beetles is, briefly, as follows: Pupae and some of the young adults hibernate under the bark of logs or trees attacked during the previous summer. Young adult beetles often emerge from infested trees in the late fall months and enter the litter and duff under the trees and spend the winter there. They come out early the following spring and attack fresh trees or logs. The males find suitable logs or trees, and then each bores a hole through the bark (fig. 6, A, a) and excavates a broad, flat chamber in the inner bark and cambium region. This chamber is known as the "nuptial chamber" (fig. 6, A, b). Several females soon join the male in this chamber and each female constructs a long tunnel known as an "egg tunnel", extending out from it (fig. 6, A, c). The pattern in which these egg tunnels radiate from the nuptial chamber is characteristic of the species. As the female extends her egg tunnel she makes small niches or pockets along each side of it (fig. 6, A, d). An egg is placed in each of these niches and covered with boring dust or frass. The eggs hatch in a few days and the young larvae begin burrowing tunnels (fig. 6, A, e) in the inner bark at approximately right angles to the egg tunnels. These larval tunnels increase in size as the larvae grow. The larvae become full grown and ready to pupate in about 3 weeks. Each larva then excavates a small oval chamber at the end of its tunnel. This is the pupal chamber (fig. 6, B). The larva then transforms to the pupal stage and later to the adult, the length of this transformation period varying with the season of the year. The young adults do not immediately emerge from the log but burrow about under the loosened bark, feeding extensively. After this period of feeding, they bore exit holes through the bark and fly away in search of a new tree or log. There are 2 generations a year in Minnesota, 1 emerging in midsummer, the other in fall and early spring.

The life histories and habits of *Ips pini* and *I. grandicollis* are rather similar. One of the chief differences is in the arrangement of their tunnel patterns. In the case of *I. pini* there are usually 3 to 6 egg tunnels, each made by a different female, radiating in a star-shape pattern from the nuptial chamber. *I. grandicollis* forms usually only 2 or 3 egg tunnels from each nuptial chamber, and these tunnels extend in a longitudinal direction very nearly parallel with the grain of the wood. This difference in tunnel pattern is important because the extent of the circumference of a log involved by a brood of *I. pini* is naturally considerably greater than that invaded by *I. grandicollis*. Since the blue-stain fungi grow most slowly in a tangential direction, the more spreading tunnels of *I. pini* usually result in a greater cross-sectional area of blue-stain infection and consequently a more rapid death of infested trees.

In May and early June 1931 many beetles were caught as they alighted upon the logs cut from a freshly felled Norway pine. Some of these were killed and fixed for histological study. Cultures were made from some, and others were dissected and examined under the microscope. Microscopic examination of these beetles frequently disclosed fungus spores adhering to the legs, wings, and other external parts. Several different kinds of spores were found, but the most abundant and most characteristic were at once recognized as ascospores

of the *Ceratostomella* found in the logs infested by the two species of *Ips*. This fungus has since been described and named by Rumbold (17) as *Ceratostomella ips*. Cultures made from the beetles, both internally and externally, yielded this fungus along with certain

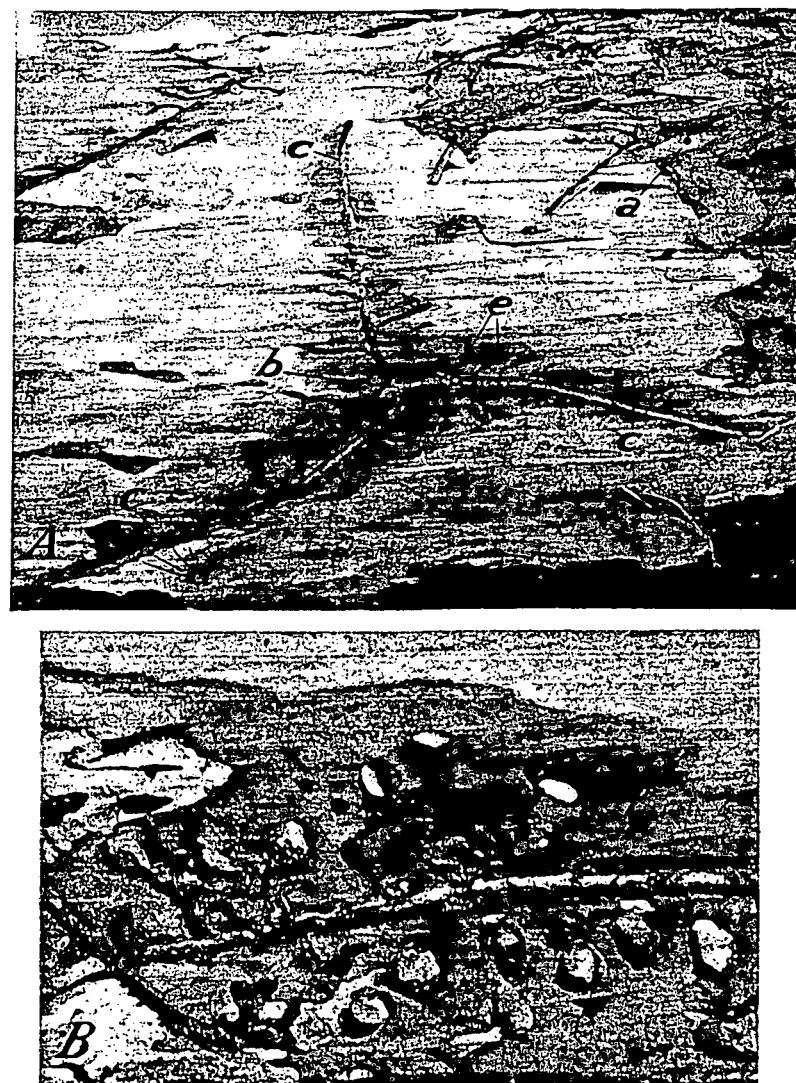


FIGURE 6.—A, Portion of a Norway pine log with bark removed to show tunnels of *Ips pini*: a, An entrance hole; b, nuptial chamber; c, 1 of 3 egg channels; d, egg niches; e, larval tunnels; B, pupal chambers and pupae of *I. grandicollis*.

characteristic yeasts. Bacteria and other fungi were frequently obtained, but *C. ips* and the yeasts were universally present. The most frequently occurring fungus other than these two was *Trichoderma koningi* Oudem. found, also, in nearly all of the old bark-beetle tunnels. The universal occurrence of these fungi in and on the bodies

of the beetles caught as they were alighting on the freshly cut logs supports the evidence that the fungi are introduced into the logs by the insects.

Among the fungi isolated from the beetles and from the stained wood adjacent to the beetles were several cultures closely resembling *Ceratostomella ips*, but bearing only conidia. The conidia were often formed on typically *Graphium*-like coremia, but on malt agar the sporophores were more commonly shortened to form tuberculate clusters. These were cultured on various media and under various conditions, but they could not be induced to form perithecia. These cultures could be identified only as species of *Graphium*. On account of the similarity of these cultures to *C. ips*, it was thought that they might be the haploid form of this fungus. (Buisman (3) has recently shown that when several strains of *G. ulmi* are mixed in culture, perithecia of *Ceratostomella* are formed.) But when several of these cultures were mixed, no perithecia were formed.

As a further check on the identity of these imperfect forms a single perithecium of *Ceratostomella ips* was placed in a drop of sterile distilled water until the ascospores oozed out of the ostiole. With the aid of a Chambers micromanipulator 15 individual ascospores were picked up and cultured. Nine of these single ascospore cultures produced perithecia in abundance and also formed a few conidia. Six of the cultures formed conidia and were similar in all respects to the *Graphium* cultures isolated previously. When each of these six cultures was grown in combination with each of the remaining imperfect cultures only conidia were formed. Moreover, when an imperfect culture was grown on agar side by side with a perfect form each retained its characteristic type of fructification. If the imperfect forms were haploid and the perfect forms diploid, there was no evidence of diploidization of the haploid form. It would appear from this evidence that the *Graphium* cultures isolated from stained wood and from the beetles were specifically identical with *C. ips* but lacked the ability to form perithecia.

Histological study of the beetles also revealed the universal presence of spores of the blue-stain fungi, both externally and internally. The beetles were killed and fixed in Dietrich's solution in a partial vacuum. They were embedded in paraffin, and sections 12 to 15 microns thick were cut and stained with the Gram-Weigert stain. This procedure revealed the ascospores of *Ceratostomella ips* adhering in clumps to various parts of the exoskeleton and also scattered throughout the intestinal tract (fig. 7). In many specimens fragments of perithecia were found, showing that the beetles feed upon these fruiting bodies. Staining reactions gave no indication that the spores were digested or otherwise injured by passage through the body of the insect. To test this point further a number of beetles were caught in sterile glass vials and held until pellets of feces were obtained. Some of these were plated on 1-percent malt-extract agar, and cultures of *C. ips* were obtained from a high percentage of them. Examination of the pellets under the microscope also disclosed the characteristic ascospores of this fungus. When the pellets were crushed and placed in hanging drops of sterile water, the spores germinated and grew normally, thus proving conclusively that they were not injured by passage through the intestinal tract of the beetles.

Cultures made from the bark and sapwood adjacent to freshly made nuptial chambers yielded *Ceratostomella ips* and yeast in nearly every

case, showing that the spores germinate soon after they are introduced. In all such chambers pellets of feces are found adhering to the walls of the chamber. The moist bark evidently furnishes sufficient moisture for germination of the spores and subsequent growth of the fungi. Although no proof can be presented, it is quite probable that spores adhering to the exoskeleton of the beetles are brushed off on the moist wood, where they would germinate and start infection.

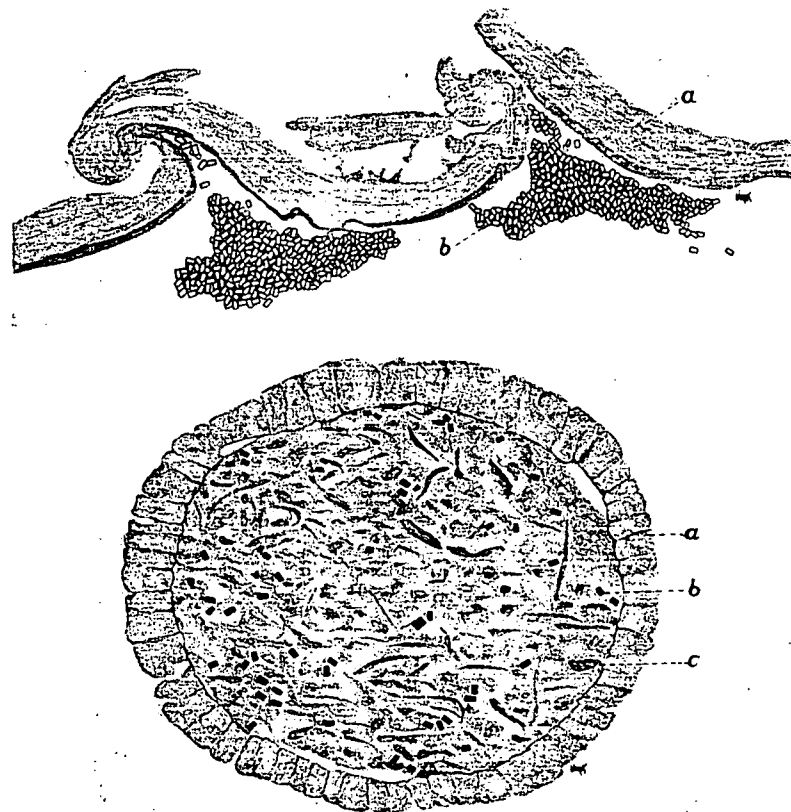


FIGURE 7.—Detailed drawings showing the spores in and on the body of mature beetles: A, Masses of ascospores adhering to the abdomen of a beetle, exoskeleton (a), masses of ascospores (b); approximately $\times 400$; B, cross section of the midintestine, showing (a) wall of intestine, (b) ascospores of *Ceratostomella ips*, (c) partly digested wood fragments; approximately $\times 400$.

Another interesting means of distributing the inoculum within the tunnel was discovered while beetles caught on the surface of the logs were being dissected. It was observed that from one to a dozen or more small mites were attached to the ventral part of the thorax or in the concave wing declivities of nearly all the beetles examined. These obviously had attached themselves to the beetles before they emerged from the old infested logs. Careful examination showed mites running about in nearly all freshly made tunnels. They were also found in great abundance in old tunnels. Apparently, they leave the beetles when they enter the log and feed on the fungi and decom-

posing bark tissue. When the beetles of the new brood are ready to emerge, some of the mites attach themselves to the beetles and are thus carried to new trees or logs. Microscopic examination of the mites taken from the beetles, as well as cultures made from them, showed that spores of the blue-staining fungus were often disseminated by them.

Of the various fungi introduced into the log by the bark beetles, the yeasts apparently develop most rapidly at first. When the outer bark is removed from a freshly made tunnel, such as the one shown in fig-

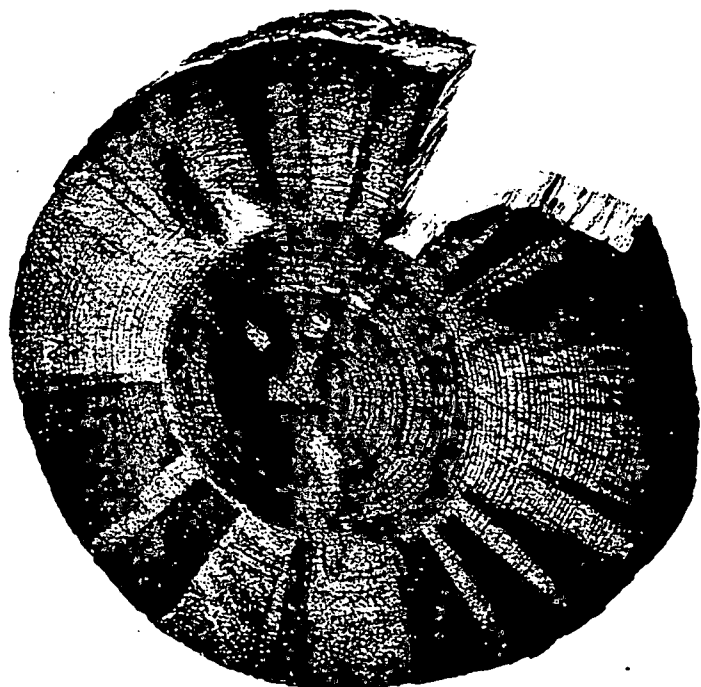


FIGURE 8.—Section cut from a log of Norway pine to show the development of blue stain in the sapwood.

ure 6, A, it is seen that the inner bark is stained brown for some distance around the tunnel. Isolations from the advancing border of this stained bark frequently yield pure cultures of yeast, while the blue-staining fungi can be found only in the bark and wood closer to the tunnel. As time goes on, the blue-stain fungi spread rapidly in the sapwood and inner bark. The rate of spread is most rapid in a radial plane, up and down the log as well as toward the center. In advanced stages stained areas appear as wedges in the sapwood when seen in cross section (fig. 8). The blue stain, however, does not affect the heartwood and its radial development ceases sharply when the heartwood is reached.

As stated above, the eggs of the bark beetles are deposited singly in small niches in the sides of the egg channel. Each egg is covered with a soft plug of sawdust. Cultures made from this sawdust plug show that it is always contaminated with yeast and other fungi. When the eggs are aseptically removed and placed on agar, it is evident they also are frequently surface-contaminated with yeast, *Ceratostomella*, and other fungi. The eggs are very delicate and easily injured, but a limited number of cultures made from surface-sterilized eggs show that they are internally sterile. As the young larvae develop and bore out a tunnel in the inner bark, the tissue surrounding the tunnel is stained brown. Yeasts can nearly always be found in the stained tissue, but such tissue is often sterile, indicating that the brown stain may be due in part to oxidation. When larvae were killed and embedded in paraffin, stained sections often revealed yeast cells in their intestinal tracts. In many cases, however, no yeast was found in the bodies of the larvae.

Shortly after the larvae pupate the blue-stain fungi begin to sporulate. The nature and amount of sporulation is more or less dependent upon the immediate environment. By this time the bark usually is becoming loose and there is a space of varying width between it and the wood. If the log is exposed to direct sunlight, its upper surface may dry out and there may be little surface growth or fructification of the fungi.

Ceratostomella ips forms two kinds of spores. Conidia are formed usually in the typical *Graphium* manner and coremia are often found in the old egg channels, the larval tunnels, or in the pupal chambers (fig. 9, A). The spores are often formed in the pupal chambers while they are still occupied by the pupae, so that the newly formed beetles are surrounded by a mass of sticky conidia. *C. ips*, however, in nature produces perithecia more commonly than conidia. The perithecia are formed frequently on the sides of the old egg channels, with their long black beaks pointing toward the center of the channels (figs. 9, B, and 10). They may be formed also in any crack in the inner bark or even completely embedded in the soft, partially decayed tissue of the inner bark. When moisture conditions are right, the ascospores ooze from the beaks of the perithecia in white sticky masses (figs. 9, B, and 10).

When transformation is complete the newly formed beetles begin feeding. They wander through the old egg channels (fig. 9, B) and also make new feeding tunnels in the inner bark. In doing this they take many spores into their digestive tract and many more stick to the external parts of the body, so that, when the beetles emerge from the log, they are thoroughly infested with fungus spores.

Although *Ceratostomella ips* was the blue-staining fungus most frequently associated with the bark beetles, another apparently undescribed fungus frequently was found that caused a stain indistinguishable from that caused by *C. ips*. When this fungus was present it usually predominated, and in many tunnels examined *C. ips* apparently was entirely absent. The fungus was first observed fruiting in the pupal chambers of *Ips pini* and was later found associated with several broods of *I. grandicollis*. Only the conidial fructification has been observed, and this is formed most frequently in the pupal chambers and in the adjacent larval tunnels. Sporula-

tion begins shortly after pupation and, by the time the mature beetle is formed, the walls of the pupal chamber are lined with a white waxy mass of conidia (fig. 11). Observations have shown that these conidia often form part of the first food consumed by the newly

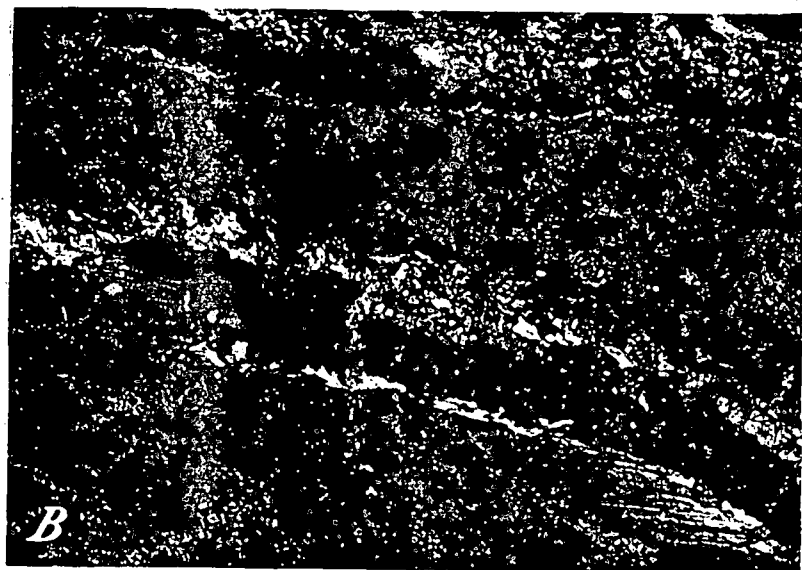


FIGURE 9.—A, *Graphium*-like coremia formed by *Ceratostomella ips* in a pupal chamber of *Ips pini*, approximately $\times 10$. B, Bark beetle (*I. grandicollis*) in an old egg channel: note the perithecia of *C. ips* lining the tunnel, with their beaks bearing glistening masses of spores all pointing toward the center of the channel. Such beetles become contaminated with the spores both internally and externally.

formed beetle. The rather large hyaline globose to pyriform conidia (fig. 12, A) are formed on the ends of short, erect palisaded sporophores, forming a compact cushion (fig. 12, B). In its general aspects this fungus resembles the so-called "ambrosia" fungi described by Hubbard (10), Neger (14), Schneider-Orelli (18), and

others and associated with the ambrosia beetles. Cultures of the fungus were obtained by picking up single conidia and also by making



FIGURE 10.—Perithecia of *Ceratostomella ips* in a beetle tunnel; note the glistening mass of sticky ascospores on the tips of the perithecia.

tissue cultures from blue-stained wood adjacent to the beetle tunnels. At least two different strains of the fungus were isolated that differed somewhat in spore size but were identical in other respects. The



FIGURE 11.—Two pupal chambers of *Ips pini* showing immature beetles surrounded by masses of spores of *Tuberculariella ips*.

spores of one strain were somewhat smaller and more globose than those of the other. There was considerable variation in size and shape of spores in any given culture. Some were distinctly globose,

while others were long and pyriform. Spores formed on agar cultures also were distinctly more globose than those formed in nature. The spores ranged from 7.9μ to 22.5μ in width and from 13.3μ to

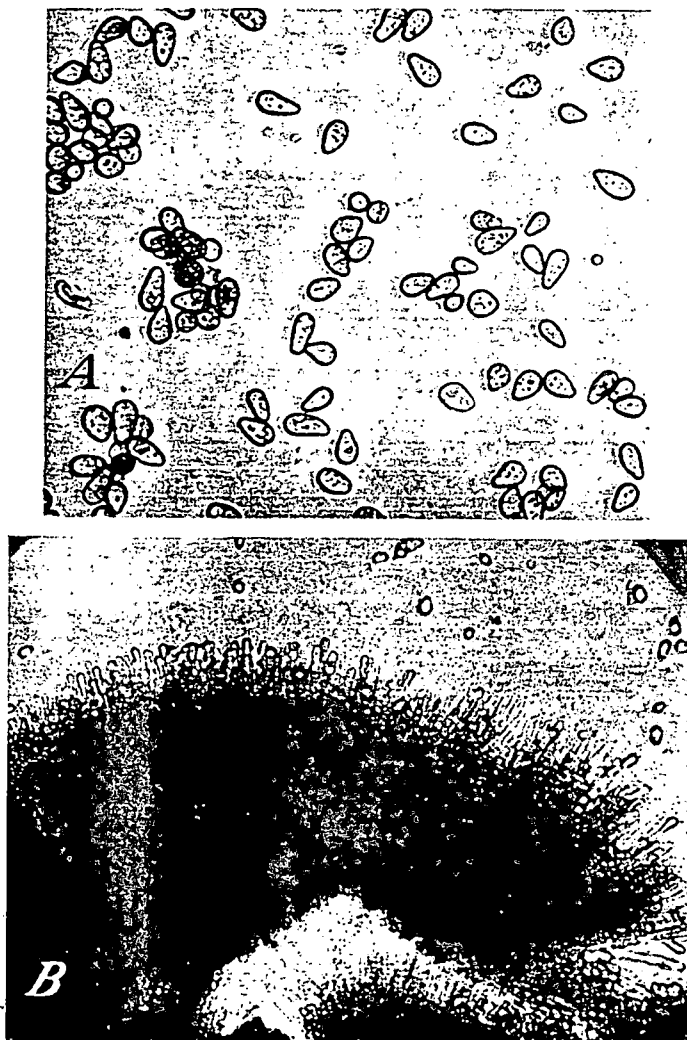


FIGURE 12.—A, Conidia of *Tuberculariella ips*; approximately $\times 130$. B, Sporodochium of *T. ips* showing the palisade arrangement and terminal position of spores; approximately $\times 50$.

23.8μ in length. The sporodochia were white and waxy, but there was no distinct mucus holding the spores together. The fungus appears to be a species of the genus *Tuberculariella* Von Höhnelt (9). Because of its close association with the two species of *Ips*, the name *Tuberculariella ips* is proposed. A brief technical description is given below.

TECHNICAL DESCRIPTION

Tuberculariella ips, n.sp.

Conidia hyaline, globose to pyriform, usually apiculate at the narrow end, ranging from 7.9μ to 22.5μ in width and from 13.3μ to 23.8μ in length, borne singly and successively at the ends of unbranched septate conidiophores; sporodochia white and waxy but not mucilaginous. Young colonies on agar colorless, later changing to black; scant gray aerial mycelium; young hyphae hyaline, old hyphae brown; mycelium 2.7μ to 6.7μ in diameter. In agar cultures conidia borne terminally on simple branches of the mycelium; sporodochia sometimes formed in old cultures.

On sapwood of *Pinus* spp. infested with *Ips pini* Say or *I. grandicollis* Eichh. at Itasca Park, Arago, Minn. Causes distinct blue stain of sapwood, and fruits abundantly in pupal chambers and larval tunnels of the insects.

LATIN DIAGNOSIS

Sporodochia alba cerea haud glutinosa; conidia hyalina, globosa vel pyriformia, plerumque apiculata ad extremitatem angustiore, 7.9μ – 22.5μ lata, 13.3μ – 23.8μ longa, ad apices conidiophorum simplicium septatorum singulatum iterum atque iterum lata.

Coloniae in "agar" cultae primum hyalinae deinde nigrescentes, mycelio aërio tenui griseo hyphis primum hyalinis deinde brunneis; mycelio 2.7μ – 6.7μ crasso, conidiis ad apices ramorum simplicium mycelii latis; sporodochiis interdum in culturis antiquis factis.

As stated above, the newly formed beetles frequently find themselves completely surrounded by masses of the conidia of this fungus. Numerous observations have shown that the beetles often feed upon these spores, frequently completely cleaning out the pupal chambers before leaving them. Dissections and histological studies also have shown large numbers of these spores in the intestinal tracts of the beetles (fig. 13). Although some of the spores appear to be destroyed by the passage, many of them evidently are not injured. A thick gelatinous cell wall usually is present on such spores stained in the intestinal tract, and the spores show no signs of injury. Microscopic examination of freshly emerged beetles shows spores adhering to the external parts of the beetles, but, unlike the ascospores of *Ceratostomella ips*, they are readily washed off when the beetles are passed through the solutions in preparation for embedding in paraffin.

By artificial inoculation it was proved that this fungus would cause typical blue stain indistinguishable from that caused by *Ceratostomella ips*. The fungus was grown also on many different kinds of media under different conditions, including irradiation with ultraviolet light, in an unsuccessful effort to induce the production of sexual spore forms. It appears, then, that the fungus is another true blue-staining fungus actively spread by these two species of bark beetles. The same fungus has been found also in association with *Ips pini* in white-pine windfalls. The factor or factors influencing the prevalence of the two blue-staining fungi and determining which one will predominate are not known.

As previously mentioned, cultural experiments showed that yeasts are universally associated with the bark beetles. They are the first to multiply extensively in the bark adjacent to freshly made tunnels. Yeasts also have been isolated from the sapwood, but here they are soon outgrown by the blue-staining fungi. The yeasts are present in great quantity in the inner bark in later stages of the beetles' development and are taken into the bodies of the mature beetles during their feeding period just prior to emergence from the log. The histological studies show yeast cells in varying amounts always

present in the food contents of the intestinal tracts of freshly emerged beetles.

On several occasions small masses of oblong hyaline conidia were found in the old tunnels of *Ips pini*. Cultures made from single spores formed brown mycelial cultures on malt agar but did not produce blue stain on wood. Examination of the mycelium showed the presence of clamp connections, and older cultures formed what

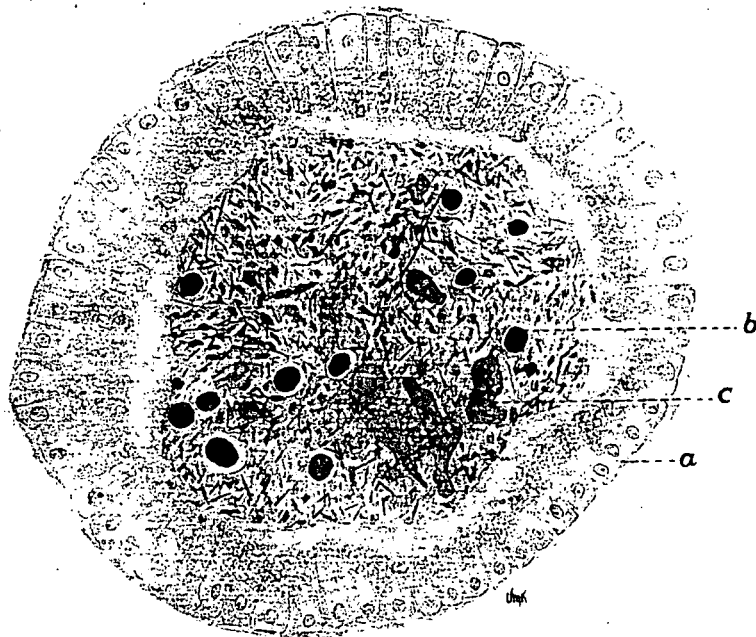


FIGURE 13.—Highly magnified section of the intestinal-tract of a mature beetle (*Ips pini*) showing the presence of viable spores of *Tuberculariella ips*: a, Intestinal wall; b, conidium of *T. ips*; c, partly digested wood fragments. Approximately $\times 75$.

appeared to be abortive poroid fruiting bodies. The identity of the fungus and its significance are not known.

In isolating fungi numerous bacterial colonies were found in the plates. These were so variable and inconsistent in their occurrence that they were considered of little significance.

In addition to the bark beetles and the mites previously mentioned, a number of insects of various types frequently were found inhabiting old tunnels. They were mainly predators or parasites of the bark beetles. Since these insects enter the logs only after the bark beetles and associated fungi have become thoroughly established, it is thought that they are of secondary importance only.

DISCUSSION

The facts presented above show quite clearly that blue-staining fungi are definitely associated with *Ips pini* and *I. grandicollis* and that they are disseminated and introduced into trees or felled logs by

these beetles. The nature of the fructification of the blue-staining fungi makes it appear highly improbable that wind is of any significance in disseminating them. The results of the controlled experiments with caged and uncaged logs, some with sealed ends and some with ends exposed, bear out this conclusion. Although the blue-staining fungi are the most obvious ones associated with the beetles, cultural studies show that other fungi are present with more or less regularity, even when other insects are absent. When one looks at the inner bark of a log at the time beetles are emerging and sees the many different kinds of fungi growing and sporulating, it is obvious that several different fungi must be disseminated with some degree of regularity. It is possible that some of these organisms are of greater significance than would be indicated by general observations. On the other hand, evidence, both cultural and histological, tends to show that the blue-staining fungi and the associated yeast fungi are predominant, both in the amount of inoculum carried by the bark beetles and in the rapidity of their development in the log.

As would be expected, other fungi develop as the disintegration of the inner bark advances and as the bark is loosened from the wood. These, without doubt, play a part in the ultimate destruction of the log and cannot be ignored completely. The most common of these "secondary" fungi is the green moldlike fungus identified as *Trichoderma Koningii*. It fruits abundantly in the old tunnels before and after the beetles have emerged. The spores are borne loose and dry in a manner favoring wind distribution, but since it has been isolated from the bodies of freshly emerged beetles, probably it also is frequently disseminated by them.

The constant association of fungi with these bark beetles naturally raises the question of the symbiotic relationships between them. As previously mentioned, Grosmann (7) has already considered this question in her study, which dealt primarily with *Ips typographus* L. and to a lesser extent with several other species of bark beetles. The blue-staining fungus associated with *I. typographus* was described by Grosmann and named *Leptographium penicillatum*. In addition, 2 or 3 characteristic yeasts were always present. Grosmann reached the conclusion that no true symbiosis existed between the beetles and their associated fungi. She concluded from her experiments that the beetles were not dependent upon the blue-stain fungi and that if any benefits were derived from the association they were all in favor of the fungus. She also concluded that the yeasts were in no way necessary for the development of the beetles and were to be considered as commensals.

Although the writers have not made extensive experiments dealing with the nutrition relations of the bark beetles and the associated fungi, observations and histological studies make it seem very unlikely that the beetles utilize the fungi extensively as food. In the examination of several hundred prepared slides of insects in all stages of development there was no indication that the fungi were digested. Yeast cells were often found in the intestinal tract of the larvae, but they showed no signs of disintegration and appeared to pass through uninjured. They were frequently entirely absent, and when present did not appear to be multiplying rapidly. No fungi of any sort were found inside the body of the pupae. In adult beetles both yeast cells and spores of the blue-staining fungi were generally

present, but they, likewise, appeared to be uninjured. Since neither of these fungi destroy wood to any appreciable extent and apparently do not multiply in the body of the insects, it is highly improbable that they digest the wood particles taken in by the insects. The feeding experiments of Grosmann (7) would tend to show that the action of the fungi on the tissues of the bark do not make it more suitable as food for the beetles. Since her experiments did not include the entire life cycle of the insects and were not carried out under fungus-free conditions, this conclusion cannot be accepted as beyond question.

One other factor, apparently overlooked by Grosmann, is the effect of the fungi on the immediate environment of the developing brood of beetles. Until it is possible to bring about the development of a brood of beetles in bark entirely free of fungi, it cannot be safely concluded that the fungi do not in some way influence favorably the development of the beetles. In the writers' experiments in which logs were protected from insects by cages and from fungus infection by sealing or spraying the ends, the bark did not separate from the wood as did that of infested logs. When the tissue of such bark as a medium for beetle development is compared with that overgrown by fungi and separating from the wood, it is readily seen that the two environments are strikingly different. Moreover, most conifers secrete resin into freshly made wounds, and in living trees such resin secretions are often able to overcome the attack of individual insects or broods. Many bark beetles are known to attack living trees most successfully when the trees are weakened by drought or other factors. It is very probable that reduced resin flow or moisture content of the inner bark may be the determining factor in such cases. Nelson⁶ has shown that the blue-stain fungi, in the absence of beetles, interrupt the flow of sap in infected trees, causing a rapid decrease in moisture content and usually resulting in death within a few months. Obviously this reduces the flow of resin. Thus beetles aided by the blue-stain fungi are able to attack certain trees which without such aid they could not successfully attack. Such benefits derived from the fungi by the beetles would certainly come within the broader concept of symbiosis, as generally used. That the fungi derive benefit from the association through dissemination by the beetles and by introduction into the tissues of the inner bark is obvious from the facts mentioned above. Their special adaptation to dissemination in this way would indicate a symbiotic relationship of long standing.

Person (16) has demonstrated recently that the inner bark of western yellow pine when fermented by the yeast associated with *Dendroctonus brevicornis* Lec., is more attractive to this beetle than the nonfermented bark. This fact is offered as an explanation of the selection of certain trees for attack by the beetle. Person believes that, in trees weakened by drought or other abnormal conditions, a limited amount of respiratory fermentation results in the production of volatile aldehydes or esters that attract a few beetles in the immediate vicinity of the trees. These introduce the yeast, which increases the rate of fermentation and results in an increased amount of the attractive substance sufficient to attract beetles from a greater

⁶ Nelson, R. M. See footnote 4.

distance. If this explanation is correct, the yeasts also must be considered as truly symbiotic with the beetles.

Buchner (2) and others have described several wood-inhabiting beetles that possess special anatomical modifications of the female definitely adapted to insure the perpetuation of symbiotic fungi by contamination of the egg either before or after oviposition. When this study was started it was suspected that some such structure would be found in the bark beetles. Careful study of many sections of the insects in all stages of development has, however, revealed no such structures. This accords with the observations of Grosmann (7) on *Ips typographus*. Transmission of the fungi from one generation to another apparently is accomplished entirely as indicated previously, namely, by means of spores and yeast cells either adhering to the external parts of the insect or passing through the intestinal tract and being expelled in the feces in newly made tunnels. Male and female beetles appear equally effective in introducing the fungi. Both yeasts and blue-stain fungi have been isolated from freshly made nuptial chambers containing only the male insect. The fungi have been isolated also from the bodies of both male and female beetles. In several instances in caged logs large patches of blue stain developed from nuptial chambers into which no females entered.

SUMMARY

A study of two species of bark beetle (*Ips pini* Say and *I. grandicollis* Eichh.) and the fungi associated with them has been made as the first part of a general investigation of the interrelations of insects and fungi in the deterioration of felled logs of Norway pine.

Experimental evidence is presented showing that these bark beetles introduce blue-staining fungi into the logs and that the fungi are rarely, if ever, introduced in any other way.

Two different blue-staining fungi were found associated with these bark beetles. The most prevalent of the two is *Ceratostomella ips* Rumbold, the fungus isolated by Rumbold from the galleries of *Ips calligraphus* and *I. grandicollis*. The second apparently has not previously been reported. It is briefly described in this paper as *Tuberculariella ips*, n.sp.

Certain cultures of *Graphium* isolated from stained wood and from the beetles proved to be identical with *Ceratostomella ips*, although they could not be made to produce perithecia. Of 15 cultures derived from single ascospores of *C. ips*, 6 formed only conidia and were identical with the *Graphium* cultures previously isolated. The remaining 9 cultures formed both conidia and perithecia. Perithecia were not produced when the 6 *Graphium* cultures were mated in all combinations.

In addition to the blue-staining fungi, characteristic yeasts were constantly associated with the beetles.

The fungi are introduced by either male or female beetles, and they begin to grow in the inner bark and sapwood soon after introduction. The yeast fungi grow more rapidly at first in the inner bark, but the blue-stain fungi spread more extensively in the sapwood.

Mites are frequently introduced into the logs by the beetles. The mites attach themselves to the underside of the thorax and in the concave wing declivities, where they are not easily brushed off.

When the beetles enter a log some of the mites leave the beetles and move about in the tunnels as they are constructed. Yeast cells and spores of the blue-staining fungi were found adhering to the bodies of the mites. The mites probably aid in distributing the spores about the beetle tunnels.

The blue-staining fungi sporulate profusely during the pupation of the bark beetles. *Ceratostomella ips* forms perithecia more commonly than conidia, but typical *Graphium coremia* are often found in the old egg channels or in the pupal chambers. The perithecia of *C. ips* are usually formed on the walls of the old egg channels with their beaks pointing toward the center of the channels. When moisture conditions are favorable, the spores ooze from the tips in sticky masses. The newly formed beetle leaves its pupal chamber and feeds extensively under the bark before emerging. In doing this it brushes against the sticky spores which adhere to the body of the beetle. Examination of the contents of the intestinal tract of beetles shows that ascospores and even parts of the perithecia are eaten. Large quantities of spores and yeast cells are found in the intestinal tracts of the beetles. These bear no signs of injury, and germination experiments show that they are still viable after passage through the body of the beetles.

The second blue-stain fungus forms masses of sticky conidia in the pupal chambers and in the old egg channels during the pupation period. These spores also adhere to the bodies of the beetles and are passed through the intestinal tract uninjured.

Yeast cells frequently are found in the intestinal tract of the larvae, where they also are apparently not injured. No fungi were found inside the body of the pupae.

Histological study of the mature beetles revealed no anatomical modification to insure transmission of the fungi to the young.

The eggs of the beetles were internally sterile, although yeast and fungus mycelium were abundant in the sawdust plugs covering the eggs in the niches.

Although no nutritional symbiosis could be demonstrated between the beetles and their associated fungi, the relationship is considered as one of true symbiosis in the broader sense. The fungi obviously derive benefit in being disseminated by the beetles and in being introduced into the inner bark of the logs or susceptible trees. The blue-staining fungi, by inhibiting the flow of sap, in all probability make living trees more favorable for beetle development, and by aiding in the decomposition of the inner bark cause it to separate from the wood, creating a more favorable environment for the development of the insect broods. Until a brood of beetles can be reared in a fungus-free log, it cannot safely be concluded that the fungi are not necessary for the normal development of the beetles.

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